

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/EP2005/002975

International filing date (day/month/year)
21.03.2005

Priority date (day/month/year)
22.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12Q1/44, C12Q1/04, C12Q1/18

Applicant
GOLDSCHMIDT GESELLSCHAFT MIT BESCHRÄNKTER HAFTUNG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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**WRITTEN OPINION OF THE
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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☐ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☐ in written format
 - ☐ in computer readable form
 - c. time of filing/furnishing:
 - ☐ contained in the international application as filed.
 - ☐ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application,
- ☒ claims Nos. 1-24,28,29 [w.r.t industrial applicability]

because:

- ☒ the said international application, or the said claims Nos. 1-24,28,29 [w.r.t industrial applicability] relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☐ no international search report has been established for the whole application or for said claims Nos.
- ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:
 - the written form ☐ has not been furnished
 - ☐ does not comply with the standard
 - the computer readable form ☐ has not been furnished
 - ☐ does not comply with the standard
- ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.
- ☐ See separate sheet for further details

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Box No. IV Lack of unity of invention

1. ☐ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:
- ☐ paid additional fees.
 - ☐ paid additional fees under protest.
 - ☐ not paid additional fees.
2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
 - ☒ not complied with for the following reasons:
see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
- ☒ all parts.
 - ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43b/s.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	19
	No: Claims	1-18,20-29
Inventive step (IS)	Yes: Claims	
	No: Claims	1-29
Industrial applicability (IA)	Yes: Claims	25-27
	No: Claims	

2. Citations and explanations

see separate sheet

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Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)
and /or
2. Non-written disclosures (Rules 43*bis*.1 and 70.9)
see form 210

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

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Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

- 1 The subject-matter of independent claim 1 and all claims dependent thereon involves the step "collecting a sample". The scope of this step covers both a method of treatment of the animal or human body by surgery (e.g. collecting swabs from internal body parts, blood samples etc) and a method of diagnosis practised on the human or animal body. Furthermore, the scope of claims 17-19,21-24,29 covers administering test therapeutic compounds to the human or animal body. This both encompasses a method of treatment of the animal or human body by surgery (administering the compounds) and by therapy (the production of therapeutic effects). As such, no opinion is given for the industrial applicability of claims 1-24,28,29 (Rule 67.1(v)).

Re Item IV

Lack of unity of invention

2 DOCUMENTS

- D1: LANGLET S ET AL: "A new gel tube method for the direct detection, identification and susceptibility testing of bacteria in clinical samples" FEMS MICROBIOLOGY LETTERS, vol. 170, no. 1, 1 January 1999 (1999-01-01), pages 229-235, XP002343236 ISSN: 0378-1097
- D2: US-A-5 098 830 (BAR-OR ET AL) 24 March 1992 (1992-03-24)
- D3: US-A-4 603 108 (BASCOMB ET AL) 29 July 1986 (1986-07-29)
- D4: DE 196 08 320 A1 (BIOSQUANT GMBH, 15344 STRAUSBERG, DE) 28 August 1997 (1997-08-28)
- D5: US 2004/048326 A1 (ROGER-DALBERT CELINE) 11 March 2004 (2004-03-11)
- D6: US 2002/031795 A1 (JAMES ARTHUR ET AL) 14 March 2002 (2002-03-14)
- D7: WEI G X ET AL: "Proteolysis and utilization of albumin by enrichment cultures of subgingival microbiota" ORAL MICROBIOLOGY AND IMMUNOLOGY, vol. 14, no. 6, December 1999 (1999-12), pages 348-351, XP002343237 ISSN: 0902-0055
- D8: LAGARDE D ET AL: "High-throughput screening of thermostable esterases for industrial bioconversions" ORGANIC PROCESS RESEARCH AND

DEVELOPMENT, CAMBRIDGE, GB, vol. 6, no. 4, 27 June 2002 (2002-06-27),
pages 441-445, XP002288681

D9: DATABASE WPI Section Ch, Week 200308 Derwent Publications Ltd., London,
GB; Class B05, AN 2003-084866 XP002288684 & JP 2002 326942 A
(NONOGAWA SHOJI KK) 15 November 2002 (2002-11-15)

D10: US 2003/199017 A1 (REYMOND JEAN-LOUIS ET AL) 23 October 2003
(2003-10-23)

D11: JAEGER K-E ET AL: "BACTERIAL LIPASES" FEMS MICROBIOLOGY
REVIEWS, ELSEVIER, AMSTERDAM, NL, vol. 15, no. 1, January 1994
(1994-01), pages 29-63, XP000490500 ISSN: 0168-6445

- 3 According to the description (p. 1, paragraph 1), the problem to be solved in the present application relates to detection of organisms in a sample without previously culturing the microorganisms. The technical solution as laid out in the present claims is to use substrates transformable by an enzymatic activity. The single general concept which can be identified as *a priori* linking the various claimed inventions is the notion that substrates transformable by an enzymatic activity can be used for detection of organisms in a sample without previously culturing the microorganisms
- 4 D1 discloses: a method for the direct (i.e. without any culturing: p. 229, column 2) detection of *E. coli* in urine samples involving contacting the sample with methylumbelliferyl substrate and detecting transformation of the substrate by *E. coli* beta-glucuronidase (p. 231; fig. 1)
- 5 D2 discloses: a method for direct detection of *C. albicans* on a vaginal swab that has not been subjected to a culturing step (claim 1, last line) involving contacting the sample with methylumbelliferyl substrate and detecting transformation of the substrate by *C. albicans* peroxidase (claim 1; c. 3-5).
- 6 D3 discloses a method for detection and identification of microorganisms directly on clinical samples using a range of detectable substrates specifically transformable by a range of particular microorganisms (c. 3, l. 4-20; c. 4, l. 64-70).
- 7 D4 discloses a method for detection and identification of microorganisms directly in

water samples using detectable substrates specifically transformable by microorganisms found in unsafe drinking water (c. 2, l. 67 - c. 3, l. 3).

- 8 in light of these documents, the above identified single general concept is not new and can thus not be the single general inventive concept as required by Rule 13.1 PCT. The present application is therefore considered not to fulfil the requirement of unity as laid down in Rule 13.1 PCT. The objective problem is therefore to provide further substrates transformable by an enzymatic activity that can be used for detection of organisms in a sample without previously culturing the microorganisms. Each of the different substrates of the present application is then a separate solution to this problem not sharing a special technical feature in the sense of Rule 13.2 PCT.
- 9 Consequently, the groups of inventions are split up as follows: 1) 2-hydroxy-4-p-nitrophenoxy-butyl decanoate / hexanoate for detecting lipase activity in skin and *Bacillus* microorganisms without previously culturing the microorganisms (claims 10-13,27 [full], 1-9,14-26,28,29 [partial]); 2) casein-resorufin for detecting protease activity in *Bacillus* microorganisms without previously culturing the microorganisms (claims 14 [full], 1-9,14-26,28,29 [partial]). No other technical features could be identified that form a technical relationship among each of the separate inventions claimed and which could be considered as a special technical feature within the meaning of Rule 13.2 PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

10 NOVELTY

- 11 The subject-matter of claims 1-18,20-29 is not new (Article 33(2) PCT).
- 12 D1 discloses: a method for **direct** (i.e. without any culturing: p. 229, column 2) detection of *E. coli* in urine samples involving contacting the sample with methylumbelliferyl substrate and detecting transformation of the substrate by *E. coli* beta-glucuronidase (p. 231; fig. 1). A kit containing a sampling tool (note: this could

be any common laboratory implement such as spatulas, tweezers, measuring devices) and a transformable substrate is therefore implicitly disclosed. The subject-matter of claims 1-3,5-8,15,16,25,26,28 is therefore not new (Article 33(2) PCT).

- 13 D2 discloses: a method for direct detection of *C. albicans* on a vaginal swab that has not been subjected to a culturing step (claim 1, last line) involving contacting the sample with methylumbelliferyl substrate and detecting transformation of the substrate by *C. albicans* peroxidase (claim 1; c. 3-5). The subject-matter of claims 1-9,15,16,25,26,28 is therefore not new (Article 33(2) PCT).
- 14 D3 discloses a method for detection and identification of microorganisms directly on clinical samples using a range of detectable substrates specifically transformable by a range of particular microorganisms (c. 3, l. 4-20; c. 4, l. 64-70). The subject-matter of claims 1-9,15,16,25,26,28 is therefore not new (Article 33(2) PCT).
- 15 D4 discloses a method for detection and identification of microorganisms directly in water samples using detectable substrates specifically transformable by microorganisms found in unsafe drinking water (c. 2, l. 67 - c. 3, l. 3). The subject-matter of claims 1-3,5-9,15,16,25,26,28 is therefore not new (Article 33(2) PCT).
- 16 D5 discloses a method for detection of *Salmonella* strains involving direct inoculation of collected samples into a culture medium containing chromogenic or fluorogenic esterase substrates (paragraphs [0021], [0028]; examples 1 and 2) (note: none of the method claims exclude the possibility culturing of the sample following step d). Importantly, the procedure of D5 does not require a culturing step in between steps c and d. The subject-matter of claims 1-3,5-8,11,15,16,25,26,28 is therefore not new (Article 33(2) PCT).
- 17 D6 discloses a method for detection of bacteria involving direct addition of a sample and nitrocoumarin detectable substrate to a culture medium (paragraphs 27-29). Like D5, no culturing step is required between steps c and d. The subject-matter of claims 1-3,5-8,11,15,16,25,26,28 is therefore not new (Article 33(2) PCT).
- 18 D7 discloses a method for detection of microorganisms of sub-gingival plaque by

detection of the transformation of resorufin-labelled casein by microbial proteases. Here, the sample is collected from a culture (p. 349, c. 2, last paragraph). However, **since the sample is not further cultured before step d**, the disclosure of D7 is prejudicial to the novelty of claims 1,2,5-9,14-16,25,26,28 (Article 33(2) PCT).

- 19 D8 discloses a method for detecting microorganisms that have a thermostable esterase involving collecting a sample from a cell culture, contacting with 2-hydroxy-4-p-nitrophenoxy-butyl decanoate, and measuring conversion thereof (p. 442, column 2). Note, there is **no** culture step between collecting the sample of microbes and contacting with the transformable substrate; **claim 1 does not exclude collecting the sample from a culture**. The subject-matter of claims 1,2,5-13,15,25-28 is therefore not new (Article 33(2) PCT).
- 20 D9 (WPI abstract) discloses a method of identifying compounds useful in the treatment of acne and rough skin involving testing the ability of a candidate compound to inhibit microbial lipase activity, which is detected by transformation of 4-methyl umbelliferyl oleate. Note, there is **no** culture step between collecting a sample of microbes and contacting with the transformable substrate. The subject-matter of claims 1,2,5-9,11,15-18,20-26,28,29 is therefore not new (Article 33(2) PCT).
- 21 By the applicant's own admissions (application, p. 16, l. 10-20), D10 discloses derivatives of 4-umbelliferyloxy-1,2-butanediol and 4-nitrophenoxy-1,2-butanediol. D10 also implicitly discloses objects that could serve as sampling tools. The subject-matter of claims 25-26 is therefore not new (Article 33(2) PCT).

22 INVENTIVE STEP

- 23 The subject-matter of claim 19 merely adds routine modification options to the subject-matter of claim 1 and is therefore obvious to a person skilled in the art. For this reason the subject-matter of said claims does not involve an inventive step in the sense of Article 33(3) PCT.

24 INDUSTRIAL APPLICABILITY

- 25 The subject-matter of claims 25-27 is considered industrially applicable (Article 33(4) PCT).

Re Item VI

Certain documents cited

- 26 The IPEA would like to point out that the priority right of the present application is probably invalid for many claims, as the priority document is demonstrably different from the present application.

Re Item VII

Certain defects in the international application

- 27 The independent claims of the present application are not in the two-part form in accordance with Rule 6.3(b) PCT. It is felt that it would be essential to use such a form in any set of amended claims as it is so unclear what the applicant considers the special technical features of the present application to be.

Re Item VIII

Certain observations on the international application

- 28 The present claims are ***in extremis*** broad in relation to the contribution to the art and several essential features appear to be missing from independent claim 1, contrary to Article 6 PCT.
- 29 Firstly, it is noted that the technical aim as laid out in the description is to provide a method that does not require time-consuming culturing steps (p. 10; p. 13, l. 5-10). However, the claims do not exclude this possibility. The sample in independent claim 1 could be obtained from a culture as in D7-D9. Alternatively, the skilled person could incorporate a culture step after contacting the sample with the substrate (step d), as in D5 and D6. Thus, the scope of independent claim 1 does not exclude methods involving a culturing step. As such, there appear to be **many** essential features missing from the claims (e.g. the origin of the sample, **direct (???)** detection of the substrate, etc).

- 30 To further compound the lack of clarity, it is not clear what the applicant means by "directly detectable" in claim 15. It appears from p. 13, l. 5-10, that this term has a special meaning. However, the term "directly detectable" should be clear from the claims **alone**. It is noted though that p. 13, l. 5-10 still does not make it clear that no culturing step takes place between steps a - e in dependent claim 1, as the technical terms used in the description (p. 13, l. 5-10) are different from the technical terms used in the claims. Step e could still be carried out whilst culturing the microorganisms. Overall, claim 15 is not clear (Article 6 PCT), particularly what the "at least one additional step" could be.
- 31 More fundamentally, there is **grave** lack of support and disclosure for the subject-matter of independent claim 1 across the whole of the claimed scope. The claims cover detecting **any** organism (i.e. including **non-cultivable** organisms) by detecting **any** enzyme activity with **any** substrate. Indeed the method is not even limited to microorganisms. However, the examples only show three specific substrates that can be used to detect specific enzyme activities in specific microorganisms without any **cultivation** step (2-hydroxy-4-p-nitrophenoxy-butyl decanoate / hexanoate for lipase activity in skin and Bacillus microorganisms; casein-resorufin for protease activity in Bacillus microorganisms). The person skilled in the art would not know from common general knowledge in combination with the present disclosure what other substrates could be used to detect other particular types of enzyme found in other particular groups of organisms. Indeed, by the applicant's own admissions (e.g. p. 3), it is customary in the art to always incorporate cultivating / culturing into methods for detecting microorganisms. Finally, for 4-umbelliferyloxy-1,2-butanediols (claim 10), no such technical teaching has been established in the application as filed. As such, the subject-matter of the present claims is considered to be **plainly** unsupported (Article 6 PCT) and undisclosed in the application as a whole (Article 5 PCT). Independently of the above reasoning, the subject-matter of independent claim 1 defines substrates in term of "a result to be achieved" (i.e. that they can detect non-cultivable organisms). As such, the subject-matter of independent claim 1 is further unclear (Article 6 PCT), **as it is not limited to the substrates, corresponding enzymes and microorganisms for which the applicant has shown this result to be achievable (i.e. those in dependent claims 13, 14).**

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CONCLUSION: Overall, the present application is extremely far away from complying with the requirements of the PCT.